(12) UK Patent Application (19) GB (11) 2 290 293 (13) A

(43) Date of A Publication 20.12.1995

(21) Application No 9411534.2

(22) Date of Filing 08.06.1994

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(51) INT CL[6]
A61K 45/06 , C07K 14/155 , C12P 21/08 , C12Q 1/68 , G01N 33/577 33/68 // (A61K 45/06 31:60 38:02 39:00 39:21)

(52) UK CL (Edition N)
C3H HK2
A5B BJA B180 B31X B31Y B317 B327 B48Y B481 B482 B58Y B586
G1B BAC BAD B103 B104 B108 B121 B200 B201 B223 B224 B301 B307 B309
U1S S1332 S1334 S1337 S2410 S2411 S2419 S2420

(56) Documents Cited
EP 0501233 A1 WO 93/11237 A1 SU 001707078 A1 US 5171841 A

(58) Field of Search
UK CL (Edition M) **A5B BJA , C3H HK2**
INT CL[5] **A61K 45/06**
ONLINE DATABASES: WPI,CLAIMS,DIALOG/BIOTECH

(54) **Preparation which inhibits the autoimmune response in HIV, or SLE, patients**

(57) A preparation which at least partially inhibits the autoimmune response in patients infected with HIV or SLE (systemic lupus erythematosus) in vivo, as determined by an autoreactive T cell proliferation assay in vitro, is described. The preparations, which may be used in the treatment of HIV(AIDS) or SLE, preferably comprise at least one non-steroidal antiinflammatory (especially aspirin) and at least one polypeptide (preferably, either a viral protein sequence having at least 50% homology with a human protein sequence, or a human protein sequence having at least 50% homology with a viral protein sequence, said sequences having up to 30 amino acids).

There is also disclosed:
(i) assay for proteins of the partial formulae DRNTQIFKTNT and DRETQISKTNT;
(ii) a monoclonal antibody, capable of binding with (i);
(iii) DNA or RNA encoding (i).

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AUTOIMMUNITY AND AIDS PATHOGENESIS
Figure 1

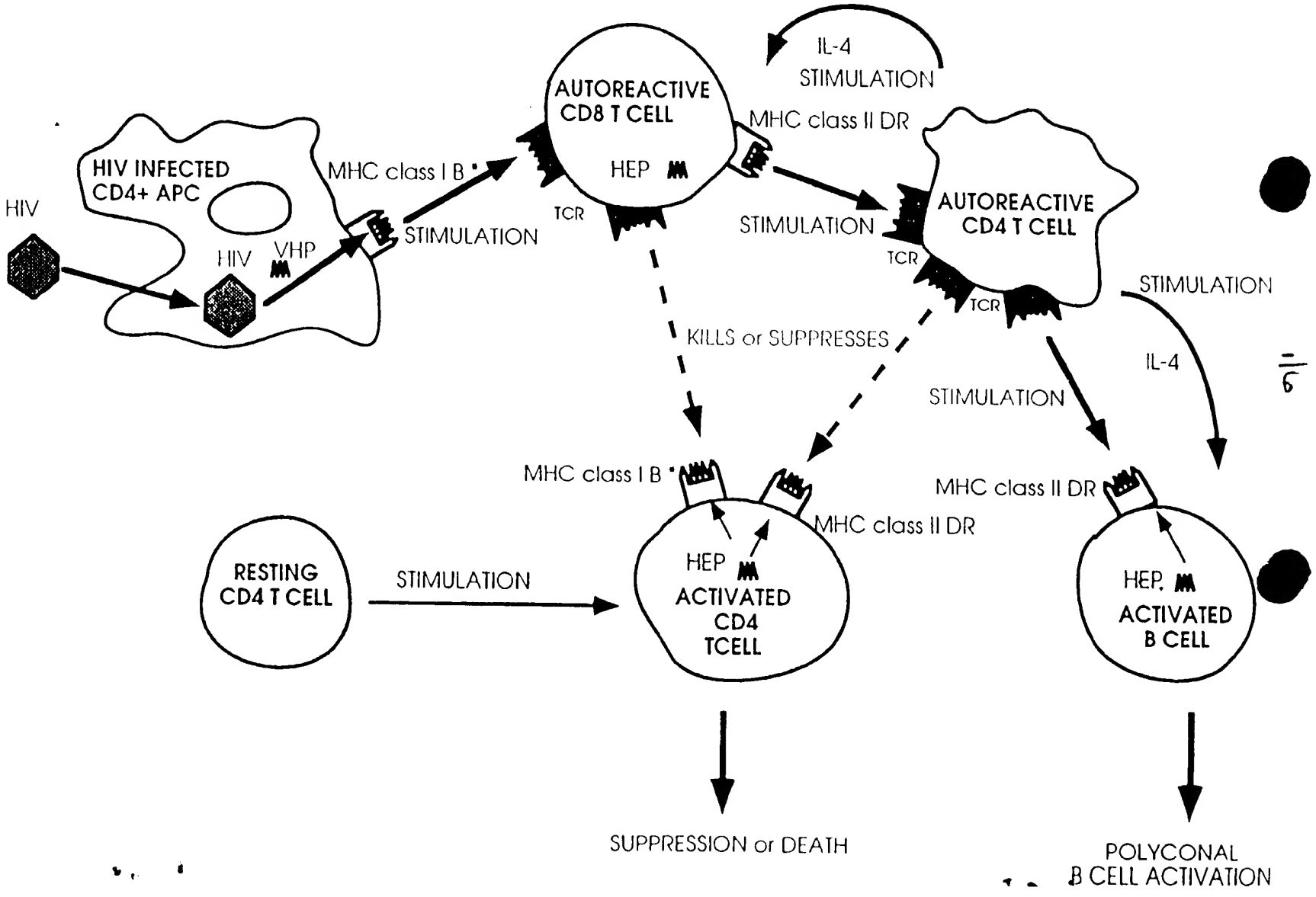

FIGURE 2

SPECIFICITY OF AUTOCYTOTOXIC T CELLS INDUCED BY VHP1

CYTOTOXICITY ASSAY

Effector Cells = PBMC + VHP1 (incubated for 4-8 hours)
Target Cells = PBMC from different donors incubated with radioactive Cr for I hour
Effector Cells + Target Cells (radiolabelled) -> incubate 4 hours = lysis and release of radioactive 51Cr

DONORS

HIV negative

EFFECTORS

Donor	HLA Phenotype	Predicted type
JMC	A1,3 B8,45 C6,7 DR3,4 DQ2,7	Sensitive to AIDS
WW	A11,36 B35,61 DR1, DQ1	Sensitive to AIDS
SW	A1,3 B35,62 DR1,4 DQ	Sensitive to AIDS

TARGETS

Donor	HLA Phenotype	Predicted type
1	A1,B8,C7,DR3 DQ2,DP3	Sensitive to AIDS
2	A31,B35,C4,DR4,DQ7	Sensitive to AIDS
3	A3,B27,C1,DR1,DQ5	Sensitive to Inflammatory Joint Disease

RESULTS

Effector: Target Ratio 10:1

% Specific Lysis	EFFECTORS		
	JMC-B8	**WW-B35**	**SW-B35**
TARGET 1-B8	30	30	9
TARGET 2-B35	30	32	10
TARGET 3-B27	7	14	2

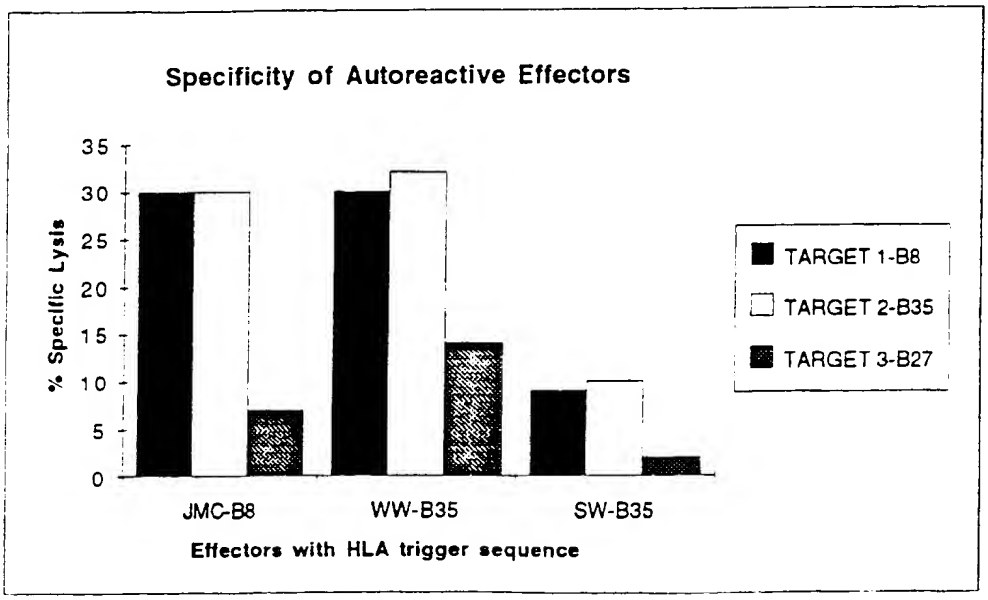

FIGURE 3 3/6

STIMULATION AUTOREACTIVE T CELL LINE WW

INDUCTION OF WW

PBMC	(HLA Phenotype)	A11 A36 B35 B61 Bw6 DR1 DQ1
PEPTIDE	VHP1	TKAKRRVVEREKR

PBMC + PEPTIDE = T CELL LINE WW

T cell lines were established by restimulating PBMC every 10-12 days with 10 µg/ml VHP1 plus additional irradiated PBMC from the same donor

SPECIFICITY OF WW

STIMULANT + WW = PROLIFERATION
(Proliferation measured by increased cpm from uptake of radioactive 3H thymidine)

Stimulant		Concentration
VHP1	TKAKRRVVEREKR	1µm
PBMC	(irradiated)	n/m
PHA		10 µg/ml
PPD		10 µg/ml
SEB	(staph enterotoxin)	0.1µg/ml

RESULTS	cpm	S.E.M.	Notes
PBMC (APC)	204	18	Background
WW (T)	150	16	Unstimulated background
Average APC,T	177		Expected for antigen specific line
PBMC	16725	390	Autoreactive proliferation
PBMC+VHP1	22721	1921	Autoreactive+antigen specific proliferation
PHA	24194	2086	Postive control
PPD	15850	632	Postive control
SEB	25698	1829	Postive control

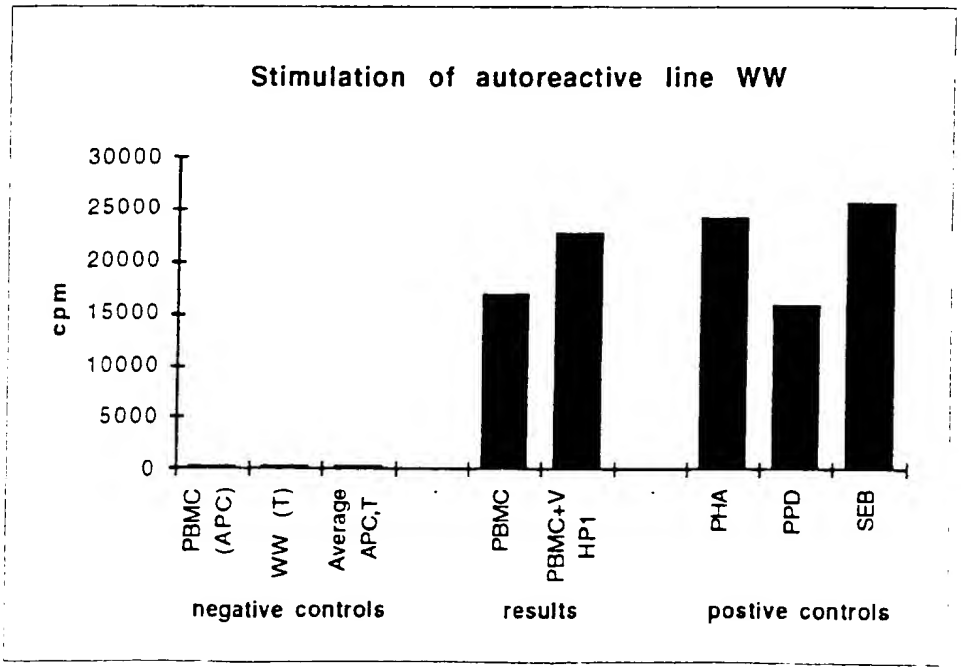

FIGURE 4

STIMULATION OF AUTOREACTIVE T CELL LINE SW

CELL LINE SW (induced with VHP1)

PHENOTYPE A1 A3 B35 B62 DR1 DR4
CD4 46.43%, CD8 34.07%

PEPTIDES

related peptides	VHP2	TKAKRRVVEREKRA
	VHP3	TKAKARVVEREKRA
	CONTROL	LEDRRAAVDTVCRA

SPECIFICITY OF SW

STIMULANT + SW = PROLIFERATION
(Proliferation measured by increased cpm from uptake of radioactive 3H thymidine)

STIMULANT=peptide (10 µm) plus irradiated PBMC

RESULTS

	cpm	S.E.M.	Notes
PBMC (APC)	314	77	background
SW (T)	302	68	Unstimulated background
Average APC,T	308		Expected for antigen specific line
PBMC	5155	94	Autoreactive proliferation
PBMC + VHP2	12272	583	Autoreactive proliferation+ antigen specific proliferation
PBMC + VHP3	7719	1183	Autoreactive proliferation+ antigen specific proliferation
PBMC + CONTROL	4554	347	Negative control peptide

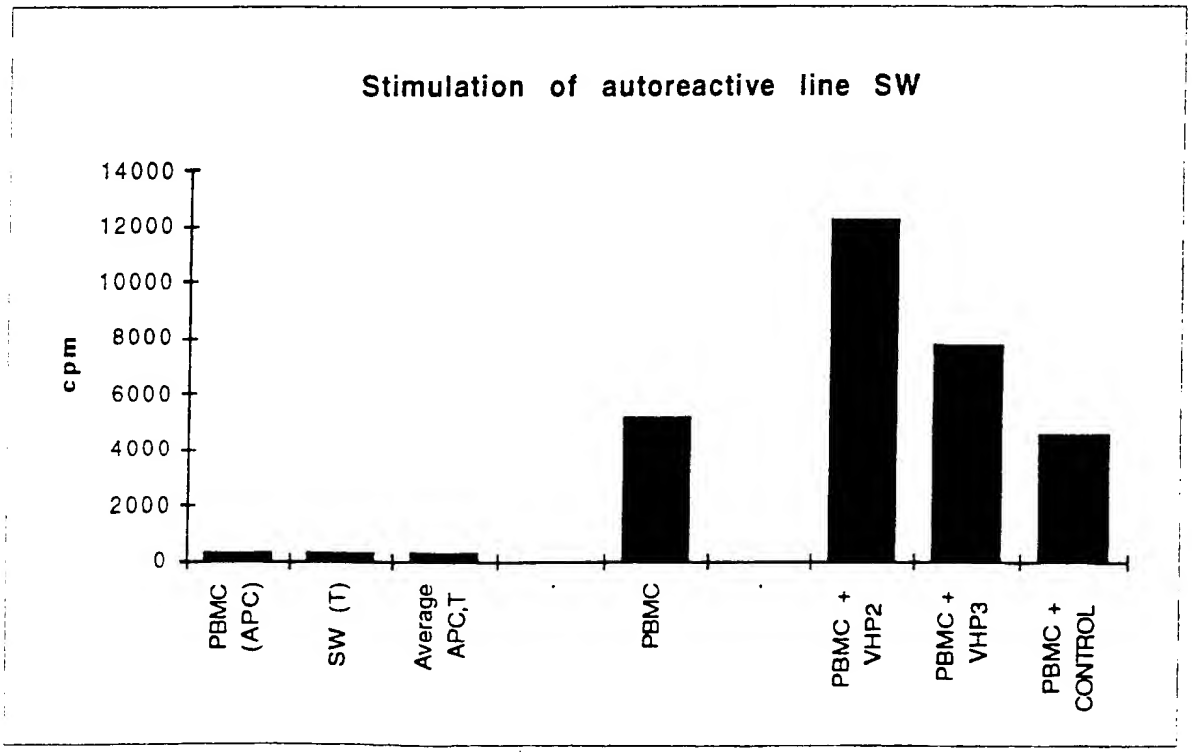

FIGURE 5

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INDUCTION OF TOLERANCE

CELL LINE WW (induced with VHP1)

PHENOTYPE A11 A36 B35 B61 Bw6 DR1 DQ1
CD4 59.11%, CD8 27.85%

PEPTIDES VHP1 TKAKRRVVEREKR

related peptides VHP4 RRVVEREKR
VHP5 VKIEPLGVAPTKAKRRVVQREKR

CONTROL RPVVSTQLLLNGSLAEEEVV
(ADP 740/23)

PROLIFERATION ASSAY
Counts after subtracting unstimulated background (APC+T)

PEPTIDE		0.1µM	1.0 µM	10.0 µM
CONTROL	0	296	-600	-1553
VHP1	0	2627	5996	1562
VHP4	0	-929	-1627	-996
VHP5	0	-988	5584	3025

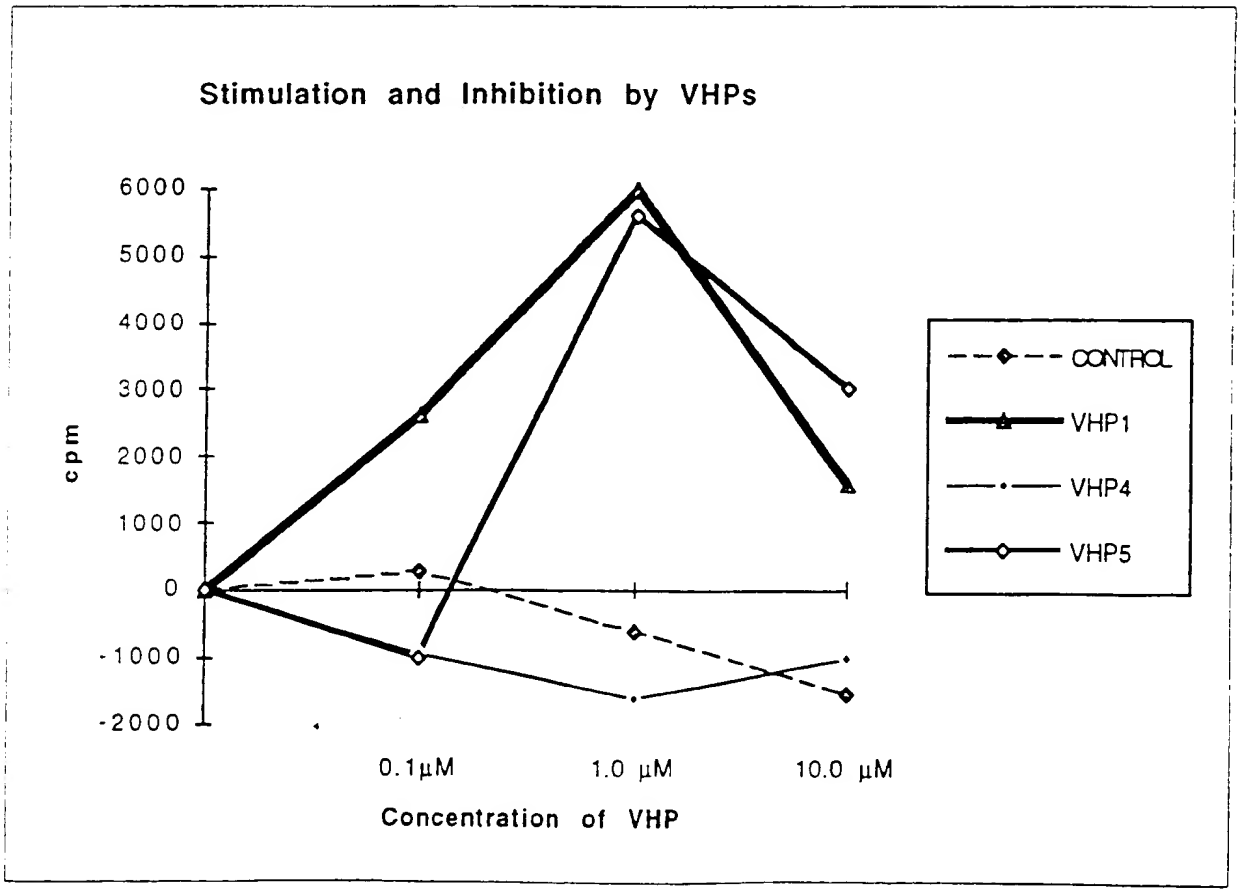


FIGURE 6

INHIBITION BY VHP1 OF AUTOREACTIVE CD4+ CLONE P2B9

CLONE

CD4+ autoreactive T cell clone P2B9 derived from cell line WW by limiting dilution

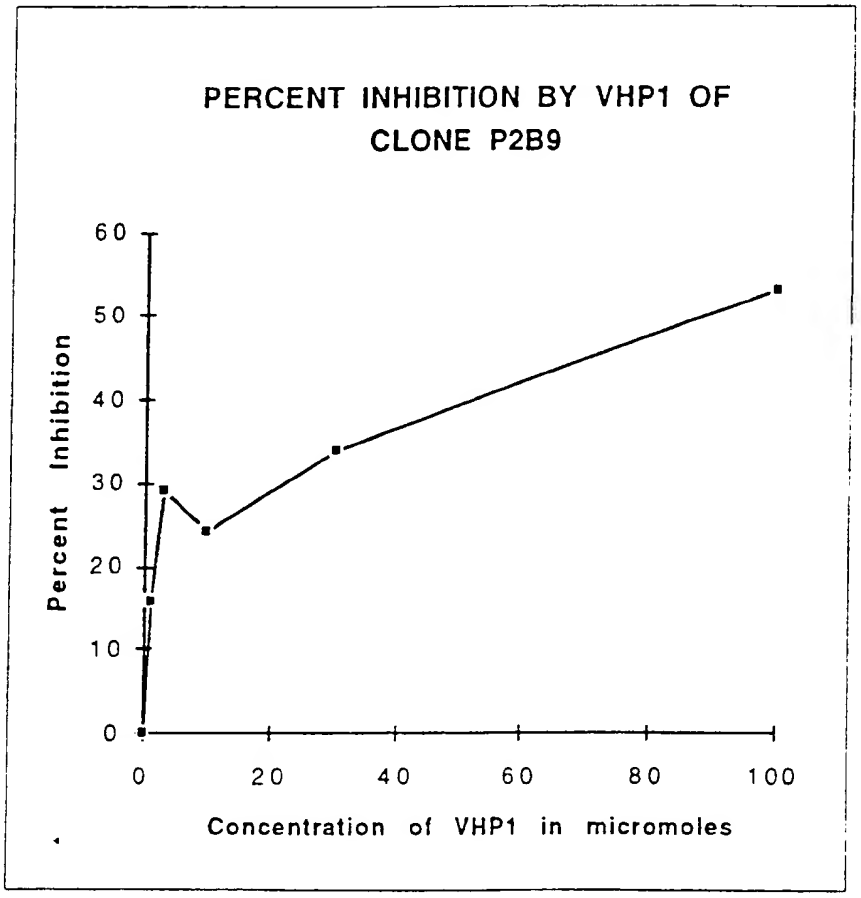
INHIBITION OF P2B9 WITH VHP1

RESULTS

PROLIFERATION ASSAY

	cpm	S.E.M	Notes
PBMC (APC)	213	17	Background
P2B9 (T)	194	26	Background
APC+ T	3686	587	Autoreactive proliferation

µM VHP1	0	1	3	10	30	100
% INHIBITION	0	15.9	29.1	24.1	33.9	53.1



AIDS PROPHYLACTICS

DESCRIPTION

INTRODUCTION

The present invention relates to substances and preparations for the prevention and treatment of AIDS and related diseases. The present invention also provides for substances and preparations for the treatment of SLE. The present invention also relates to tests for the susceptibility of an individual to AIDS, to predict the rate of progression to AIDS after HIV infection, to substances for use in such tests, to methods for the preparation of substances for use in such tests, to methods for the preparation of substances for use in such tests, and to kits for performing such tests. As used herein, the various titles, and other such headings are intended for guidance, and should not be construed as limiting on the present invention

AIDS (Acquired Immune Deficiency Syndrome) is a disease, in susceptible individuals, resulting from chronic infection by Human Immunodeficiency Virus (HIV). It is characterised by a complete breakdown of the immune system, which allows opportunistic infection by microorganisms which might otherwise never cause disease. AIDS is widely accepted as being caused by HIV but the precisely how HIV infection leads to AIDS is still a matter of active scientific investigation **(1, 2, 3)**. A number of investigators have described autoimmunity related to HIV infection but have not established that HIV induced autoimmunity directly causes AIDS **(4, 5, 6 ,7)**. The present invention is based on the discovery of the molecular mechanism of HIV induced autoimmunity which leads to AIDS. The present invention provides for preparations which prevent HIV induced autoimmunity which lead to AIDS by the induction certain types of immunosuppression or immune tolerance.

Current research into developing a vaccine to prevent AIDS is based on attempting to increase immune responses to the HIV but has so far failed to produce an effective product **(8)** whereas the present invention is based on selectively decreasing immune responses to the HIV. A number of drugs have been developed for the treatment of AIDS (mainly anti-viral drugs similar to, 3'-azido-3'-deoxythymidine, AZT), but none have been demonstrated to prevent the development of AIDS after HIV infection.

The present invention is based on the discovery of parallels between HIV induced disease and autoimmune disease such as systemic lupus erythematosus (SLE). Both SLE and HIV disease in man display polyclonal B cell activation **(9, 10, 11)**, autoantibody production including anti-histone antibodies **(12,13,14)** an association between HLA-B8 and disease progression **(15,16,17,18,19,20,21)** and immunosuppression leading to *Pneumocystis carinii* infection **(22,23)**. It is also of interest that cross

reactivity between a retroviral protein and a human protein is implicated in autoantibody production in SLE(**24**).

Most of the drug therapies used in patients with SLE are empiric. A number of problems exist in designing and carrying out drug studies in patients with SLE. Disease expressions and their severity vary widely from patient to patient making it difficult to study the effects of any drug in a homogeneous population. Furthermore, disease activity in any one patient may fluctuate markedly over varying periods of time, making it difficult to distinguish a drug action from the natural course of disease.

Non steroidal anti-inflammitory drugs (NSAIDs) such as aspirin are prescribed periodically to control low level chronic immune activation in SLE.(**25**) In general, NSAIDs reduce immune activation by inhibition of the cyclooxygenase-peroxidase enzyme pathway. This pathway catalyses the conversion of arachidonic acid to a group of prostaglandins including prostaglandin E_2 (PGE_2). In vivo, the conversion of arachidonic acid to PGE_2 initiates a range of activation signals to the immune system. In vitro, PGE_2 has been shown to strongly inhibit T suppressor cells resulting in activation of T helper cells and it has been speculated that the inactivation of T suppressor cells by PGE_2 may be an important process in autoimmune disease such as SLE. Aspirin (a NSAID) is a particulary useful inhibitor of immune activation because it perminantly inactivates cyclooxygenase by acetylating the enzyme (other NSAIDS tend to reversibly inactivate cyclooxigenase). The effect lasts until the enzyme has been replaced by natural turnover and can take up to seven days depending on the tissue. The great advantage of aspirin over other more potent immunosuppressive drugs is that its toxic side effects in man are mild and well established.

Depletion of CD4+ cells by a variety of mechanisms has been often cited as the central step in the immunosuppressive process which leads eventually to the development of AIDS. It has been demonstrated that CD4+ depletion can arise from direct viral infection of CD4+ cells (**26**), from targeting of CTL to uninfected CD4+ cells which have bound gp120 (**27,28**), through the action of either HIV-induced autoantibodies (**9**) or autoreactive CTL specific for activated uninfected CD4+ T cells (**29, 30**). The observation that HIV-infected chimpanzees suffer HIV induced CD4+ T cell depletion, but do not generate autoreactive CTL and do not go on to develop AIDS (**30**), suggests that CD4+ depletion alone is not a sufficient condition for AIDS. I believe that an autoimmune response including polyclonal B cell activation with associated autoantibody and autoreactive CD8+ CTL is a necessary condition for the development of AIDS. Indeed, patients that show early marked immune activation develop AIDS more rapidly

(31,32). HIV-like viruses which infect their natural hosts but do not induce polyclonal immune activation do not give rise to an AIDS-like disease(33).

MHC Class I molecules on the surface of cells present a selection of peptides from the cell interior (by forming MHC/peptide complexes) to passing immune T cells for analysis. MHC Class I molecules on the surface of infected cells present foreign peptides derived from the infecting microorganism, and these MHC/ foreign peptide complexes trigger the immune cells to attack. The molecular trigger is the binding of a specific T cell Receptor molecule (TCR) on the surface of a immune T cell to the MHC/foreign peptide complex on the infected cell.

MHC Class I molecules are expressed on most cell types and are mainly recognised by CD8+ T cells. The MHC Class I allele B8 is associated with several human autoimmune diseases: systemic lupus erythematosus (SLE) Addisons disease, chronic active hepatitis, coeliac disease, dermatitis herpetiformis, Graves' disease, myasthenia gravis, scleroderma, Sjogrens syndrome, and Type 1 diabetes.(15) MHC B8 is also associated with rapid progression to AIDS (18,19,20,21). It is also significant that the largest sequence divergence between chimpanzee MHC and human MHC occurs at the MHC Class I B alleles (34) and chimpanzees do not develop AIDS after HIV infection (35,36).

I have discovered a pattern of associations between HLA phenotype and AIDS on reviewing studies published by various authors (**Table 1**): HIV infection sensitivity appears to be confered by HLA-A2, A32 (A19), B51 (37,38,39); fast progression to AIDS after HIV infection is confered by HLA-B8, B35, DR1 and DR3 (18,19,20,21,96-101), HIV infection resistance appears to be conferred by A69 (A28), B44, B52 , B72 (B70) and DR4 (37,38,39,40,41,42) and long term survival after HIV infection appears to be conferred by A24 , A32, B40 (B4), B44 ,B57 and DR7 (21,43,44,45,46,47). There is also an association between HLA-B27 and inflammatory joint disease leading to AIDS in HIV infected people (48,49). Kaposi's Sarcoma appears to be associated with DR5 in HIV+ male homosexuals. SLE sensitivity appears to be associated with B8 DR1 or DR3 and resistance with B7 (50).

When a peptide derived from a virus, in a complex with certain types of MHC, is seen as sufficiently foreign to induce an immune response but has sufficient sequence homology with a human peptide to trigger T cells bearing TCRs which also recognise self peptide/MHC complexes, there is a serious risk of a chronic autoimmune response. This type of molecular mimicry has been postulated for the induction of a number of autoimmune diseases including multiple sclerosis. Adenovirus type 2 contains a peptide

sequence with 50% homology to human myelin basic protein which may induce a cross reactive autoimmunity when presented on MHC Class II DR2 molecules, which could explain the production of antimyelin autoantibodies, autoreactive CTL and clinical disease (**51,52**).

HIV-induced autoimmunity may also be due to mimicry since the virus encodes a number of protein sequences with varying degrees of homology with human sequences (**53**). Autoantibodies to human heat shock proteins and histones are detected in HIV infection (and SLE) (**4,9,13**) which may be the the result of this type of homology. A cytotoxic autoantibody with specificity for histone H2B and which reacts with an H2B histone-like protein in HIV infected individuals progressing to AIDS has been described (**13**).

I predict that an HIV sequence, to be significant for the induction of an autoimmune response associated with AIDS, is likely to fulfill the following criteria: have a high degree of homology (50% or greater) with a human sequence over 9-10 amino acid sequence (size for MHC Class 1 presentation (**54**)), have the ability to form an immunologically active epitope and particularly with HLA haplotypes associated with progression of AIDS, and be conserved over all viral isolates of HIV but different from an equivalent SIV sequence.

I propose that the sequence TKAKRRVVEREKR at position 498 to 510 (conserved C4 region) at the C-terminus of HIV gp120 (a predicted alpha-helical region) (**55**) is a good candidate for an epitope for the induction of autoimmunity. It is an immunodominant region of gp120 in man and accounts for up to 70% of the total antibody response generated to the virus in some individuals although the antibodies are non virus-neutralising (**56**). A peptide derived from this region is also a dominant MHC Class-I restricted epitope for the induction of CTL (**57**). The sequence is conserved in all isolates of HIV-1 and HIV-2 against a background of a high mutation frequency and is even more stable than the CD4 binding site (**58**). The sequence also appears to be a recent addition to the virus as it is coded by direct repeats of a putative transposable element (**59**).

I have named the group peptides derived from HIV sequences which have homologies with human protein sequences, Viral Homologous Peptides or VHP. The corresponding group of peptides derived from endogenous human protein sequences which the virus mimics, Human Endogenous Peptides or HEPs.

THE INVENTION

NSAIDs and the prevention of AIDS

It is an aspect of this invention that Non-Steroidal Anti-inflammitory Drugs, for example aspirin (Acetylsalicyclic acid), can be used alone or in combination with other agents to slow down the development of AIDS in an HIV infected person. The recommended dose is to administer between 100mg and 1000mg of aspirin once a day after HIV infection has been confirmed (or to administer up to the maximum recommended daily dose for any other NSAID)

Induction of immune tolerance with peptides and the prevention of AIDS

An HIV initiated autoimmune process mediated by VHPs in combination with population of autoreactive T cells gives rise to AIDS. Selective immunosuppression (for example by induction of T cell anergy with peptides) to switch-off the autoimmunity induced by HIV should prevent AIDS.

The present invention provides a vaccine against AIDS comprising a toleragenic dose (significantly larger than the threshhold dose sufficient to stimulate an immune response) of VHPs or HEPs or any combination thereof, or peptides similar thereto, such as the homologous sequences identified in **Table 2 or Table 3** in the presence or absense of NSAID therapy. The prefered route of administration is oral, in combination with NSAID therapy although it is equally possible to achieve tolerance by intra venous or intramuscular administration by standard methods. It is an aspect of this invention that an oral synthetic peptide vaccine can be administered to prevent AIDS in HIV infected people by the induction of high dose oral tolerance to the VHP and, or, HEP sequences.

Peptides used for the toleragenic vaccine may be synthesized using a solid phase method using Fmoc chemistry known to those skilled in the art. They are then cleaved from the resin and deprotected using trifluoromethane sulphonic acid. Peptides are desalted on a P-10 column in PBS (pH 7.3) and purified by HPLC. Homogeneity of the peptides can be demonstrated by reverse-phase HPLC. The purity of the peptides can be demonstrated by sequencing by automatic Edman degradation using gas phase sequence.

The prefered composition of the vaccine should be between 10 and 50 milligrams of a peptide or combination of peptides listed in **Table 2** or **Table 3** dissolved in distilled water to final concentration of 1mg/ml. Up to 50mls of the freshly made solution should

be given to an HIV infected person once day for twenty days. Those skilled in the art be aware of similar procedures to induce tolerance to autoreactive responses in animals by oral administration of small peptides derived from cross reactive antigens **(60,61,62,63,64).**

Possible similarities between VHP peptide and sequences in human proteins, were investigated using computer searches of the SwissProt protein sequences database (Release 25). I have now established that the pathogenic activity of HIV is as a result of the similarity between VHP1, a part of HIV gp120/41 coat protein and certain self peptides (HEPs). VHP1, an HIV derived peptide, has the sequence TKAKRRVVEREKR (13mer - occurring at position 498-510) and is apparently the most important. VHP1 has a 70% sequence homology to an evolutionarily conserved important human protein called Ezrin (at positions 324-337) **Table 4.**

In addition to Ezrin, other human (self) proteins also possess a degree of primary sequence homology with VHP1: these include Heat shock protein 89, NK-TR protein, T complex protein, Adrenergic receptor Type 2, Creatine Kinase, Trypsin inhibitor, Aldehyde Dehydrogenase, Opioid Receptor (kappa), Glycine Receptor (Alpha-2 chain) and Ryanodine receptor. Mouse Histone H2B (very similar to human Histone H2B) also has homology. (see **Table 2**).

Ezrin, a human tubulin binding protein, is found in the cytoplasm of T cells and is phosphorylated by tyrosine kinase during T cell activation. Ezrin is also known as P81, Cytovillin or Villin-2, is a protein of 585 amino acids. The homology extends over a predicted alpha-helical region of ezrin and is adjacent to the tyrosine 353 phophorylation site (P). Ezrin is part of a family of ezrin related proteins, which includes ezrin, radixin, moesin and merlin. All these proteins are related in the region that shows similarity to gp120, but ezrin has the most significant homology with 9/13 identities. Although ezrin behaves as a soluble protein in T cells and appears to be diffusely cytoplasmic by immunofluorescence staining, it is believed that the critical population of ezrin is that which is associated with the submembraneous cortical cytoskeleton. **(65)** It may also be important for the autoimmune pathology of AIDS that ezrin is also found localized to microvillar actin microfilament cores in the brush border of the intestinal epithelium and also in neurons. In gut epithelium ezrin is involved in changes in cell membrane morphology on stimulation **(66)**.

VHP1 has 50% homology with a peptide derived from human heat shock protein Hsp89b (Hsp90) which forms a significant fraction of peptides eluted from HLA-B27**(67,68,69)** . This molecular mimicry could explain the inflammatory joint disease

seen in HIV infected patients with an HLA B27 phenotype (**48,49**). The VHP1 also has homology with histone H2B(**70**). It is interesting that the homology is at the exposed N terminus of histone H2B, a region which is the target for autoantibodies in SLE (**72,73**) VHP1 also has homology with NK-TR protein, a protein which has sequences related to both ezrin and histones which is expressed in Natural Killer cells (**74**)

The sequence homologies of heatshock proteins and histones with VHP which are targets for autoreactivity in autoimmune disease suggests that this sequence is important for HIV induced autoimmunity. Although ezrin has not been associated in any reported autoimmune disorder, its important role in cell structure and its presence in T cells, the gut and brain makes it a likely target for autoreactivity to explain the diverse tissue disorders seen in AIDS patients.

I propose that the presentation of VHP peptide by MHC Class I of HIV infected cells breaks the tolerance of the host immune system to self peptides such as those derived from ezrin, heat shock proteins and histones (and other HEPs) presented on activated T and B cells. VHP triggers an autoimmune process in HIV infected individuals which could progress through the following steps (**Figure 1**)

1. HIV infected CD4+ Antigen Presenting Cells (APCs) present VHP on MHC I B* (MHC I B alleles carrying the trigger sequence DRNTQIFKTNT-see later).
2. Complexes of VHP and MHC I B* on APCs interact with a cross reactive TCR and induce MHC Class I B* restricted activation of a population of autoreactive CD8+ T cells. These autoreactive CD8+ T cells kill or suppress any activated CD4+ T cells expressing MHC1 B* / HEP complexes
3. The activated autoreactive CD8 cells also express complexes of HLA DR and HEPs
4. HLA-DR restricted activation of autoreactive TH2 CD4+ T cells could be induced by the autoreactive CD8+ T cells.
5. The autoreactive TH2 CD4+ cells then could suppress or kill activated CD4+ T cells presenting complexes of HEPs and HLA-DR.
6. The autoreactive TH2 CD4+ T cells would also produce IL-4 which stimulates B Cells and the autoreactive CD8 T cells.
7. Stimulated B cells express complexes of HEPs with HLA-DR , and could be activated further by the autoreactive CD4+ T cells to polyclonally produce antibodies including autoantibodies.

In summary, I conclude that VHP, at very low physiological concentrations, induces a chronic autocytotoxic response in HLA phenotypes associated with fast progression to AIDS (B8 or B35, DR1 or DR3) which eventually destroys the immune system.

Other peptides for the prevention of AIDS and SLE by induction of immune tolerance

DNA and RNA has been isolated from some SLE patients who have not been HIV infected which is homologous to the p24 gag/pol junction of HIV I **(75,76)** and some SLE patients also produce antibodies to p24 gag protein from HIV-1**(77)**. I propose that peptides (up to 25 amino acids in length) derived from protein sequences translated from these DNA and RNA sequences can be used to tolerise both SLE patients and HIV infected patients to prevent disease progression **(Table 3)**.

Prognostic test for AIDS susceptibility.

What is not well recognised by the public, but generally recognised by the skilled person, is that not all HIV infected (HIV+) individuals go on to develop AIDS - a significant proportion of the population of HIV+ individuals develop AIDS only many years after HIV infection and a smaller proportion will never develop the classic symptoms of AIDS. Given that treatments for HIV+ individuals are usually toxic, expensive, and time consuming, it would be extremely useful to be able to distinguish between individuals who were more, and individuals who were less susceptible to developing AIDS so that the patient receives appropriate drug therapy. The present invention provides a solution: a prognostic test using monoclonal antibodies or PCR specific for key residues on MHC Class I B alpha-1 domain which correlates to susceptibility or resistance to AIDS to broadly predict the rate of progression to AIDS.

I have established that sensitivity to AIDS is linked with particular MHC phenotypes. Each person expresses up to twelve different MHC haplotypes which makes up the MHC phenotype of the individual. MHC molecules are split into two groups, MHC Class I and MHC Class II. The MHC Class I group is split into three sub groups: A B and C. The MHC Class II group is also split into three subgroups: DP, DR and DQ. There are a number of related genes in the population for each subgroup and every individual expresses two genes (or haplotypes) for each sub group (one from each parent) making up to twelve different MHC haplotypes in the MHC phenotype. A number of factors including the unique combination of MHC molecules in each patient probably determines the precise rate of progression to AIDS.

Murine monoclonal antibody LA45 (raised against the human tumor cell line HUT 102) defines an activation induced antigen on human lymphocytes. LA45 recognises a site at

position 61-62 (Arg Asn) (**78**) on MHC Class I alpha 1 domain a sequence which interacts with TCRs (**79**). The LA45 monoclonal antibody binds to the following haplotypes of MHC Class I B: B7, B8, B14, B18, B35, B38, B39, Bw42 and B51. By contrast, LA45 does not bind to the following haplotypes of MHC Class I B: B13, B27, B37, B40, Bw41, B44, Bw46, B47, B49, B52, Bw57, Bw58, Bw60, and Bw62. I have observed that the HLA-B alleles associated with rapid progression to AIDS, B8 B35 and express the LA45 site, and that the alleles associated with slow progression to AIDS: B44 B52 do not express the LA45 site (**80**). This led me to investigate sequences in the alpha-one domain for associations with susceptibility or resistance to AIDS. Although the LA45 antigen has been previously described, its importance in determining susceptibility to AIDS has gone completely unrecognised, the research in question being part of research in the very much broader field of immunology.

My analysis of protein sequences in the alpha-1 domain of MHC-1 reveals a significant sequence association in the haplotypes which correlate with rapid progression to AIDS (**Table 5**). HLA- B8, B35 and B51 are all associated with rapid progression to AIDS and all contain the sequence: -D R N T Q I F K T N T- which occurs at position 60-70 in the alpha-1 domain. HLA-B53 and B78 also carry the same sequence in the same position in the alpha-1 domain. B53 is common in Africa and is associated with resistance to malaria (**81,82**). B78 is also a common allele found in African populations (**83**). I suggest this sequence (which interacts both with the bound peptide and the TCR) is an important trigger sequence for the induction of an autoimmune response associated with AIDS. (I shall identify HLA alleles coding for this sequence *trigger+*).

HLA alleles associated with HIV infection resistance and slower progression to AIDS have a different sequences in this region. The sequence D R E T Q I S K T N T appears to be an important sequence for conferring resistance both to HIV infection and AIDS after HIV infection. Chimpanzee MHC Class I alpha-1 domain does not contain the sequence: D R N T Q I F K T N T associated with sensitivity to AIDS but has sequences similar to the human haplotypes which confer resistance to AIDS (**84,85**). Analysis of the third hypervariable region of HLA DR reveals only a weak pattern of sequence association with either HIV infection sensitivity and rapid progression to AIDS or HIV infection resistance and slow progression to AIDS.

I propose that the pattern of HLA Class I alpha one domain sequences and the sequence homologies of VHPs provides important information to explain AIDS in terms of an SLE-like autoimmune disease. From the reported associations between HLA and HIV disease it is clear that HLA Class I and HLA Class II alleles are both involved in disease. This relationship is reflected in the clear pattern of HLA Class I alpha one domain

sequence associations and in the weaker pattern the sequences of the third hypervariable region of HLA-DR. I propose that the trigger sequence DRNTQIFKTNT on HLA-I B molecules is a shared epitope important in conferring susceptibility to AIDS. Patterns of HLA-DR sequences in the third hypervariable region have been observed in susceptibility to rheumatoid arthritis (**86**) which has supported the hypothesis that shared epitopes on HLA molecules are important in autoimmune disease (**87**). The HLA-DR association in AIDS appears to be weaker.

The critical positions in HLA Class-I B appear to be residue 62 which interacts with the TCR and residue 66 which interacts with the antigenic peptide. Sensitivity to AIDS is determined by arginine at position 62 and phenylalanine 66 while resistance to AIDS can be confered by only changing position 62 to glutamic acid and position 66 to Serine. Position 66 also contributes to the P2 specificity pocket for selective peptide antigen binding(**88,89**). Analysis of the sequences of peptides eluted from HLA-B8 and B7 suggests that K499 and K501 of VHP may be important for HLA binding (**90,91,92**)

The distribution of HLA alleles in the population is likely to have an effect on the observed HLA associations and it significant that Italian studies identify HLA-B35 (which is more prevalent in Mediterranean populations) with rapid progression to AIDS while northern European studies report HLA-B8 which is more prevalent in northern European populations. Over half the Caucasian population express alleles associated with faster progression to AIDS which correlates with the observed proportion of patients progressing to AIDS in long term cohort studies (**93**). The prevalence of HLA-B53 and B78 in African populations suggests that HIV infection will have serious consequences in this group while the very low frequency of the susceptibility epitope in the Japanese population suggests that HIV infection will have less serious effect. (**94**)

Individuals who are not sensitive to AIDS have different MHC-Class I B molecules which do not express the DRNTQIFKTNT sequence in the α-1 domain. They may also express other sequences on MHC Class I A, B and C which select for a family of T cell receptors which do not cross react between VHPs and HEPs. In addition VHPs or HEPs may not be presented correctly in association with the type of the MHC Class I in resistant individuals to trigger HIV induced autoimmunity.

Thus, in a first aspect, the present invention provides a method for the diagnosis of AIDS susceptible individuals, comprising bringing a biological sample from the individual into contact with an antibody recognising an MHC Class I B molecule associated with AIDS sensitivity, but not capable of recognising an MHC Class I B molecule associated with AIDS resistance, or vice versa, and assaying for binding of the antibody.

It will be appreciated that the preferred antibody to recognise AIDS sensitivity is one which is specific for DRNTQIFKTNT, especially the region containing RN. It will also be appreciated that the preferred antibody to recognise AIDS resistance is one which is specific for DRETQISKTNT, especially the region containing RE. However, other antibodies will also be useful where they recognise an epitope of this region, or other characteristic area. In addition, now that it has been appreciated that it is MHC Class-I that is important, it will be understood that, from the information given, it will be possible to identify diagnostic antibodies which recognise MHC Class-I haplotypes confering sensitivity to AIDS and not MHC Class I haplotypes confering resitance, as well as vice versa, so that the tests of the present invention can be either positive in the event of sensitivity or in the event of resistance, as desired, although it is preferred to provide a test positive for sensitivity, such as where the LA45 antibody is used.

The LA45 antigen cannot be detected on resting T cells, and can only be detected on activated T cells. Further, it appears that the LA45 antigen is a dominant epitope, thereby enabling antibodies to be readily raised against it.

Thus, in accordance with a further aspect of the present invention, there is provided a method for producing a monoclonal antibody producing cell line, wherein the monoclonal antibody is specific for AIDS-sensitive MHC Class I B haplotypes and does not bind AIDS-resistant MHC I B haplotypes, the method comprising tolerising a mammal, with resting T cells prepared from at least one individual having an MHC Class I B haplotype associated with AIDS-sensitivity and, thereafter, exposing the immune system of the animal to an immunogenic dose of activated T cells from at least one individual known to have an MHC Class I B haplotype associated with AIDS-sensitivity, the individual in each case preferably being the same, both for the tolerising and the immunogenic doses, and thereafter preparing the cell line in accordance with known techniques.

Although the tolerising and immunogenic doses, as specified above, are described as being from an individual, it will be appreciated that each of the doses may be from more than one individual. However, it is generally preferred that one and the same individual is used for the preparation of both the resting T cells and the activated T cells.

The activated T cells may be activated by a known technique for example. One technique involves exposing the cells to a dose phytohaemagglutinin, or other suitable toxin.

Furthermore, as stated above, it is preferred that the antibody to detect AIDS sensitivity is raised by against the DRNTQIFKTNT sequence. The preferred haplotype for preparing

the monoclonal antibody producing cell line is A1, B8, DR3. MHC-1 B8 contains DRNTQIFKTNT at position 60-70 and is strongly associated with disease progression to AIDS.

Monoclonals produced by the above technique may be screened against resting T cells, in order to eliminate those monoclonals recognising antigens on resting T cells, thereby to leave cell lines which produce monoclonal antibodies against antigens which are only present on activated T cells.

Screening may also be performed against activated T cells of haplotypes known to be resistant to AIDS, as a negative screen, and against activated T cells of haplotypes know to be sensitive to AIDS, as a positive screen.

The present invention also provides monoclonal antibodies, and cell lines producing them, when prepared by the methods described above.

Diagnosis, as defined above, may generally be performed by any known methods of assay involving specific antibodies. Many types of assay are known, and all that is necessary in the present invention is that an indication be provided as to whether the antibody is binding anything in the sample. Given that the antibody is specific for MHC Class I B obtained from the activated T cells from AIDS sensitive (also referred to herein as "AIDS susceptible") individuals, and to denatured MHC Class I B obtained from such individuals, then binding will indicate that the individual from whom the example is taken is susceptible to AIDS. Appropriate prophylactic and/or therapeutic action can then be taken if desired and/or required.

Prior to the assay, it is generally preferred to prepare the sample in some way, given the LA45, for example, fails to recognise the LA45 antigen in resting T cells. Where antibodies are used which cannot bind the appropriate antigen in resting cells, then appropriate preparation of the sample will be required.

Suitable preparation of the sample may involve activation of the T cells by exposure to suitable toxin, for example, or may involve lysis and/or other degradation to expose the antigen. In a kit for home use, suitable enzymes may be supplied, or a simple system for osmotic rupture provided.

Typical assay methods are ELISA (enzyme-linked immunosorbent assay), sandwich assays and competitive assays, all of which are well known and described in the art.

For example, in ELISA, it is common to use an enzyme which can act on a substrate to yield a coloured product. A typical enzyme is horseradish peroxidase, which can be linked either to the detecting antibody, or to another antibody. ELISA can be combined with a sandwich assay, such that an antibody is immobilised on a support, and the sample is then introduced to immobilised antibody. After a suitable period, the sample is washed off, and the second antibody is introduced to the support. This assay can take a number of forms. In one form, the antibody for detecting AIDS susceptible haplotypes is immobilised on the support, so that only that part of the sample which contains MHC of an AIDS susceptible haplotype is bound to the support. An enzyme linked antibody is then introduced to recognise, for example, activated T cells which have become bound to the support. This antibody can then be washed off and any remaining antibody assayed by introduction of substrate for the enzyme.

Alternatively, the sample may, in some way, be immobilised to the support, such as be means of a polyclonal antibody specific for T cells, and the enzyme-linked, diagnostic antibody, specific for AIDS susceptible haplotypes, can be introduced directly with the sample, if desired. The support is then washed to remove any unbound sample and unbound antibody, and the substrate for the enzyme introduced. If colour is detected, then this indicates that the diagnostic antibody has bound to the sample, and that the individual is AIDS susceptible.

The particular advantage of the above technique is that it can be very simply provided in the form of a kit for use at home. Although the result will not necessarily be quantitative, it will be qualitative, and is sufficient to provide a very rapid yes/no diagnosis.

Accordingly, the present invention further provides a diagnostic kit, comprising antibody specific for AIDS susceptible haplotypes of MHC I B, together with means for assaying binding of the antibody to a sample.

It will also be appreciated that the type of labelling can vary from enzymes, and may be in the form of a radioactive tracer, for example, the only limitation being that binding of the antibody can be assayed.

It will be appreciated that, because a particular amino acid sequence DRNTQIFKTNT is characteristic of AIDS susceptibility, then a suitable chosen DNA primer for a PCR (polymerase chain reaction) test can also be used to diagnose AIDS-susceptibility or AIDS resistance. Such a test forms a further aspect of the present invention.

Suitable oligonucleotide primers for the PCR test of the present invention are illustrated in **Table 6** A number of primers may be required for any one test, or a single characteristic primer may be used.

The following Example serves to illustrate the invention only, and should not be contrued as limiting it in any way.

EXAMPLE 1

IN VITRO MODEL OF VHP-INDUCED AUTOIMMUNITY IN HUMAN CELLS FROM DONORS (WITH AIDS SUSCEPTIBLE MHC Class I) AND INHIBITION OF AUTOIMMUNITY WITH A PEPTIDE VACCINE.

MATERIALS and METHODS

Peptides

Peptides used in this study were synthesized using a solid phase method using Fmoc chemistry. They were cleaved from the resin and deprotected using trifluoromethane sulphonic acid. Peptides were desalted on a P-10 column in PBS (pH 7.3). Homogeneity of the peptides was indicated by reverse-phase HPLC. Peptides were sequenced by automatic Edman degradation using gas phase sequence and were shown to be >95% pure. Sequences used in this study are shown below:

CODE	SEQUENCE
VHP1	TKAKRRVVEREKR
VHP2	TKAKRRVVEREKRA
VHP3	TKAKARVVEREKRA
VHP4	RRVVEREKR
VHP5	VKIEPLGVAPTKAKRRVVQREKR
CONTROL	LEDRRAAVDTVCRA
ADP740/23 Control	RPVVSTQLLLNGSLAEEEVV

Donors

Donors positive for B8 or B35 *(Trigger+)*

Donor	A	B	C	DR	DP	DQ
FM	1,3	8	7	3	nd	nd
SW	1,3	35,62	nd	1	nd	1
WW	11,36	35,61	nd	1	nd	1
JMC	1,2	8,45	6,7	3,4	nd	2,7
Target 1	1	8	7	3	3	2
Target 2	31	35	4	4	nd	7

Donors negative for B8 or B35 *(Trigger-)*

Donor	A	B	C	DR	DP	DQ
TM	3,28	7,14	nd	6	nd	nd
JF	2	17	3	6	nd	1
KB	1	7,14	7	4,11	nd	7
ML	2	44,52	5	2	nd	1
Target 3	3	27	1	1	nd	5

Generation of short-term cultures

PBMC were isolated from 8 HIV seronegative individuals. Four donors FM, SW, WW, JMC *(Trigger+)* where positive for either HLA B35 or B8 and four donors TM, JF, KB, ML *(Trigger-)* where negative for HLA B35 or B8. PBMC (10^6/ml) were incubated in the presence of VHP1 or control peptide at 100 ng/ml for 4-8h. Irradiated autologous PBMC without VHP1 stimulation served as controls.

Cytotoxicity

VHP1 induced short term cultures of autoreactive T cells (Effector Cells) were assayed for cytotoxic activity against autologous PHA T cell blasts stimulated with 5 µg/ml PHA for 4-5 days at a concentration of 10^4 cells/well in the absence of either control or VHP peptide (Target Cells). 1-2 x 10^6 Target Cells were labeled with 250mCi of ^{51}Cr in 0.3 ml of medium for 1h at 37•c. Target cells were washed 3x in medium, diluted to 10^5 or 10^4 cells/ml and 100ml of cell suspension added to each well of a V-bottomed 96-well microtitre plate. Effector Cells were added to the Target Cells at Effector:Target (E:T) ratios from 50:1 to 6.25:1. Cultures were incubated for 4h at 37•c and supernatants were harvested and counted for ^{51}Cr release. Percent cytotoxicity is calculated using the formula : 100x (experimental release - spontaneous release)/(maximum release-spontaneous release). Spontaneous release and maximum release were determined by incubating target cells in medium or 1.0% Triton x-100 respectively. Results where calculated as means ± SD of triplicate cultures and a positive result was scored above 20% specific lysis. The cytotoxicity assay was repeated for allogeneic target cells from donors (Target-1, Target-2 and Target-3) and the percent specific lysis scored at an Effector:Target ratio of 10:1.

Induction of autoreactive T Cell Lines by VHP.

PBMC were isolated from healthy HIV seronegative donors WW (A11,36; B35,61; Bw6; DR1,2; DQ1) and SW (A1,3; B35,62; DR1,4). PBMC (10^6cells/ml) were stimulated with 10 µg/ml VHP in triplicate 24-well cultures with RPMI 1640 containing 10% AB+ human serum, 2mM L-glutamine, 100U penicillin and 100 µg/ml streptomycin. The cultures were restimulated every 7-10 days with peptide (VHP) and irradiated (30 Gy) autologous PBMC. Following restimulation cultures were fed every 3-4 days with complete medium containing rIL-2 at 20U/ml Lines were tested for cytotoxicity 7 days after the last restimulation. Prior to proliferation assay T cell lines were rested in the absence of IL-2 overnight.

T cell Proliferation Assay

The proliferation assays was performed by culturing T cell lines (10^4 cells/well) in U-bottomed 96-well microtitre plates for 72h. Separate cultures of the T cell lines were incubated with fresh irradiated (30Gy) autologous PBMC (10^5 per well) PBMC in the presence and absence of 1µM VHP for 3 days at 37•c. Three positive control cultures of the T cell lines were stimulated with 10µg/ml PHA, 10µg/ml PPD and 0.1µg/ml SEB (Staph. Enterotoxin) respectively to determine maximum proliferation. The two negative controls were an unstimulated culture of T cell line and irradiated PBMC under the same conditions. During the final 6h cultures received 1mCi/well of [^{3}H] thymidine. Cells were harvested onto glass fibre filters by a semiautomatic harvester and the incorporation of labeled thymidine was quantitated by liquid scintillation spectrometry. The results are expressed as cpm of the mean for triplicate cultures. T cell lines were rested in the absence or r-IL2 for 24h prior to assay.

RESULTS

Induction of autoreactive cells by VHP

Short term cultures of PBMC from donors positive for B8 or B35 (*trigger+*) stimulated with VHP produced Effectors which showed significant autocytoxic responses against PHA activated autologous T cell targets in the Cr release cytoxicity assays. Short term cultures PBMC from donors positive for B8 or B35 stimulated with control peptide and short term cultures PBMC from donors negative for B8 or B35 (*trigger-*) did not produce these autocytotoxic Effectors.(**Table 7**)

Further Cytotoxicity assays with allogeneic PHA activated T cell Targets were then performed with the Effectors derived from short term cultures of PBMC from donors of

either HLA B8 or B35 (*trigger+*) stimulated by VHP. The Target Cells were from donors with either B8 B35 (*trigger+*) or B27 (*Trigger-*) to establish the HLA specificity of the cytotoxic cells (**Figure 2**).

B8 and B35 both have the trigger sequence in the alpha-one domain whereas B27 has a different sequence. HLA-B8 JMC Effectors recognised both B8 and B35 Targets but not B27 Targets. HLA-B35 WW Effectors recognised both B8 and B35 Targets but not B27 Targets with the same specificity as the HLA-B8 JMC Effectors. HLA-B35 SW Effectors showed a similar pattern of specificity in that they recognised both B8 and B35 Targets but not B27 Targets but the total cytotoxic activity was lower. I conclude that VHP is presented by HLA-B8 and B35 of the Antigen Presenting Cells (APCs) to Effector T cells in the short term cultures of PBMCs. I also conclude that HLA-B8 and HLA-B35 Effector T cells are recognising a common epitope on HLA-B8 and HLA-B35 of the Targets which is absent on HLA-B27. The common epitope is likely to be a combination of the common HLA-I B trigger sequence, DRNTQIFKTNT, (present in the alpha one domain of both B8 and B35 but not on B27) plus a HEP, (a self peptide homologous to VHP).

An autoreactive T cell line was established from donor WW (HLA-B35 phenotype) by stimulation with VHP. The line was a mixed population of 59% CD4+T cells and 28% CD8+ T cells. A proliferation assay was performed to measure to level of autoreactive proliferation in the T cell line and the level of additional VHP specific proliferation (**Figure 3**). T cell line WW responded vigorously to autoantigens presented on autologous irradiated PBMC to similar levels as the positive control stimulatants, PHA, PPD, or SEB. The data also shows an additional VHP specific proliferation in addition to the autoreactive response.

VHP1 specific proliferation was investigated further with the T cell line established with PBMC from donor SW (**Figure 4**). Cell line SW (CD4 46% CD8 37%) induced with VHP1 was tested for proliferation to VHP2 (VHP1 with an additional COOH terminal A), a modified peptide VHP3 (R502 had been replaced by A and the COOH terminal A had also been added) and a control peptide (the same number and type residues as VHP1 but in a different sequence). VHP2 stimulated cell line SW efficiently, VHP3 stimulated the line poorly and the control peptide did not stimulate the cell line at all. We conclude that peptides related to VHP1 stimulate VHP1 induced autoreactive T cell lines and that the addition of a COOH terminal A does not effect the activity of VHP1. However, the substitution of R502 for A in VHP3 significantly effects the activity of VHP.

Specific stimulation was further investigated with T cell line WW induced by VHP1 (**Figure 5**). Autoreactive cell line WW was stimulated with VHP1 and different VHP peptides related to VHP1 to determine the critical residues. VHP4 (VHP1 without the TKAK N terminus) did not stimulate the line where as VHP5 (which includes the VHP1 sequence but with a 506 replaced with Q and additional flanking sequences) stimulated the line to a similar extent as VHP1. At concentrations higher than 10μM, both VHP1 and VHP5 showed declining proliferation indicating the beginning of tolerance induction. The results indicate that the TKAK sequence is critical for the activity of RAP.

Inhibition by the induction of tolerance with VHP1 was investigated with a VHP1 induced CD4+ autoreactive clone produced by standard limiting dilution methods. The clone was exposed to increasing concentrations of VHP1 and an absolute decline in autoreactivity was observed. 50% inhibition was observed above 100μM VHP1. (**Figure 6**)

DISCUSSION

I conclude that VHP1, at low concentrations, induces autocytotoxic cells in donors of HLA phenotypes associated with sensitivity to developing AIDS (B8 or B35, DR1 or DR3). VHP1, at low concentrations, is inactive in donors who do not carry for MHC haplotypes associated with sensitivity to developing AIDS. The data indicates that the critical region in VHP1 for induction of autoreactivity is the TKAKR sequence. VHP1 induces both autoreactive CD4 and CD8 cells which is consistent with my proposed mechanism for the induction of AIDS. The inhibition, with high concentrations of VHP1, of an autoreactive T cell clone induced by VHP1 provides support for the invention to induce tolerance to VHP1 *in vivo* as a method to prevent HIV induced autoreactivity and AIDS.

TABLE 1

Publications reporting an association between AIDS and HLA phenotype

REF	INFECT SEN	INFECT RES	HIV+FP	HIV+LTW	HIV+OI	HIV+KS	HIV+AIDS	HIV+PGL	HIV+CD4 dec	STUDY SIZE
18			8.1 35.2 35.3 AH							190
19			A1B8DR3							32
20			A1C7B8DR3		B35 DR3	B35 C4 DR2 DR5				Review
			B35 C4 DR1 DR5							
21			A1C7DR3						A24 SLOW	108
48			B27 Inflam Jnt Dis							9
49			B27 Inflam Jnt Dis							
96			B35							144
97			B35		DR2	DR5 (B8 DR3)				174
98			B35							126
99			B35							
100			B35							
101			B35							
18			B8 B35 DR3 DR1							190
102			C4BQ0							285
50			**SLE B8 DR3,**	**SLE B7**						417
43			DR4 DQB302	B4 B57					B4 SLOW	801
44				A32 B4 C2						585
45				DR7/DRB10702			C4B-L			114
				DQA10201						
46			DR5	B44 C5 weak						31
103					CW7	DR1 DR14 DQ1	DR1			266
104						DR5 B44 A23 B49				21
105					DR3	DR5 DR2				35
106								DR5 (DR2)		20
107						DR5				
108						DR5 (B8 DR3)				39
109								DR5		69
47						B35 C4 DR1 DQ1			DR1 FAST (DR7)	120
37	A19	A28 B70								263
38	A2	B52 DR4					DR3 DQ2			76+62
39	B51 DR5	B52 B44								147
40	no DR5 assoc									46
41	dec DR5 assoc					A28				411
42		A69								

( ) Negative association

Ref=Reference, Infect Sen= infection sensitive, Infect Res =infection resistant

HIV+FP=Fast progress to AIDS after HIV infection, HIV infected long term well, HIV infection plus opportunistic infections,

HIV+KS=HIV infection plus Kaposi Sarcoma, HIV+AIDS= HIV infected with AIDS,

HIV+PGL= HIV infection plus persistent generalised lymphadenopathy, HIV+CD4 dec=HIV infection plus rapid CD4 decline

Ref 110: Nomenclature for factors in the HLA system

TABLE 2

VIRAL HOMOLOGOUS PEPTIDES (VHPs) AND HUMAN ENDOGENOUS PEPTIDES HEPs)

VIRAL HOMOLOGOUS PEPTIDES (VHPs)

(* = ANY OR NO AMINOACID)

CODE	PROTEIN SOURCE	SEQUENCE (Q = Q Alternate)
VHP0	gp120 POSITION 484-511	* * * * * * * * * * * * * * * T K A K R R V V E R E K R *
VHP1	gp120 POSITION 498-510	T K A K R R V V E R E K R
VHP2	gp120 POSITION 498-511	T K A K R R V V E R E K R A
VHP3	gp120 POSITION 498-511	T K A K A R V V E R E K R A
VHP4	gp120 POSITION 502-510	R R V V E R E K R
VHP5	gp120 POSITION 488-510	V K I E P L G V A P T K A K R R V V Q R E K R
VHP6	gp120 POSITION 484-510	K Y K V I K I E P L G I A P T K A K R R V V E R E K R
VHP7	gp120 POSITION 496-505	* * T K A K R R * *

HUMAN ENDOGENOUS PEPTIDES (HEPs)

CODE	PROTEIN SOURCE	SEQUENCE
HEP1	EZRIN/CYTOVILLIN/P81	T E K K R R ET V E R E K E
HEP2	HUMAN Heat Shock Protein 89b	R R V K E V V K R
HEP3	NK-TR protein	R K H K R R RK V K R SK K R
HEP4	HISTONE H2B	T K A OK K D G K K R - K R
HEP5	TYROSINE KINASE PRECURSOR	R A M R R Y L E R
HEP6	T-COMPLEX PROTEIN	A M A V R R V L K R DL K R
HEP7	ADRENERGIC RECEPTOR/2	R R R AH V T R E K R
HEP8	CREATINE KINASE	T R A E R R E V E R
HEP9	TRYPSIN INHIBITOR	L L A K R M K V D R E E R
HEP10	GLY RECEPTOR ALPHA-2 CH	K K K F V D R A K R
HEP11	ALDEHYDE DEHYDROGENASE	R R S V E R A K K
HEP12	OPIOID RECEPTOR (KAPPA)	K A K R K V V
HEP13	RYANODINE RECEPTOR/MUSCLE	T K A Q R D V I E

TABLE 3

HEPs DERIVED FROM RNA SEQUENCES FROM SLE PATIENTS

(RNA HOMOLOGOUS TO HIV P24 GAG-POL DNA SEQUENCES)

HUMAN ENDOGENOUS PEPTIDES (HEPs)

CODE	SEQUENCE
HEP14	D L P S C K G
HEP15	Q W T F L R A G K R
HEP16	I C L P A R E A
HEP17	G L F S E Q P R
HEP18	S A F R Q G K P V D F S Q
HEP19	G R R Q Q L R L R T Q D R
HEP20	N C I L T S I R S F
HEP21	I E D N N S V S E G R T D R T V S
HEP22	K T T T P S Q N A G P I E L Y L N F H Q I I S
HEP23	D T V L S V L R S E T E L L S S T
HEP24	N D L M E V K I Q F Y R S C V L R R
HEP25	W K L R Y
HEP26	S I G P A F
HEP27	D G V V V F Y
HEP23	L L A L R K V H W L P F Q E G R
HEP24	E K S T G F P C R K A D
HEP25	P L A S L A G R Q I

TABLE 4

Homology between HIV-1 gp120 C4 alpha helical domain and human EZRIN alpha helical domain

Cleavage site
gp120-gp41
X X

SEQUENCE																												
	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	508	509	510	511
gp120	K	Y	**K**	V	I	**K**	I	E	P	L	G	I	A	P	**T**	K	A	**K**	**R**	**R**	V	**V**	**E**	**R**	**E**	**K**	R	A
			*			*									*			*	*	*		*	*	*	*	*		
EZRIN	E	E	**K**	H	Q	**K**	Q	L	E	R	Q	Q	L	E	T	E	K	**K**	**R**	**R**	ET	**V**	**E**	**R**	**E**	**K**	E	Q
	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	332	333	334	335	336	337	338

TABLE 5

HLA protein sequences and HLA association with AIDS

Sequence Position & Orientation T=TCR, P=Peptide, O=Other *=variable Ref: 79		MHC1 phenotype alpha-one domain 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 T P O T T P O T P P O T P O T P P O T * * * * * * *		MHC11 phenotype DRB1 third hyper variable region alpha-two domain 70 71 72 73 74 T P O T TP * * * * *
HIV INFECTION SENSITIVE	A2	Y W D G E T R K V K A H S Q T H R V D		
	A19	Y W D Rgl E T R N V K A Qh S Q Tl D R V D		
	B51	Y W D R N T Q I F K T N T Q T Y R E N		
HIV+ FAST PROGRESSION TO AIDS	B8	Y W D R N T Q I F K T N T Q T D R E S	DR3	Q K R G R
	B35	Y W D R N T Q I F K T N T Q T Y R E S		
(Malaria resistance)	B53	Y W D R N T Q I F K T N T Q T Y R E N	DR1	Q R R A A
(frequent HLA type in Africa)	B70	Y W D R N T Q I F K T N T Q T D R E S		
HIV INFECTION RESISTANT	A69/A28	Y W D R N T R N V K A Q S Q T D R V D	DR4	Q K R A A
	B44	Y W D R E T Q I S K T N T Q T Y R E S		D E R A A
	B52	Y W D R E T Q I S K T N T Q T Y R E N		Q R R A E
	B72/B70	Y W D R E T Q I S K T N T Q T Y R E S		Q R R A A
HIV+ LONG TERM WELL	B7	Y W D R N T Q I Y K A Q A Q T D R E S	DR7	D R R G Q
	B40/B4	Y W D R E T Q I S K T N T Q T Y R E S		
	B44	Y W D R E T Q I S K T N T Q T Y R E N		
	C2	Y W D R E T Q K Y K R Q A Q T D R V N		
	A32/A19	Y W D Q E T R N V K A H S Q T D R E S		
	B57	Y W D G E T R N M K A S A Q T Y R E N		
	A24	Y W D E E T G K V K A H S Q T D R E N		
HIV+ KAPOSI'S SARCOMA			DR5	D R R A A D E R A A
HIV+ Inflam joint disease/AIDS	B27	Y W D R E T Q I C K A K A Q T D R E D		
CHIMPANZEE SEQUENCES	A1	Y W D Q E T R S A K A H S Q T D R V D	DRB10302	D R R G Q
	A2	Y W D E E T R S A K A H S Q T D R V D	DRB10305	D R R G Q
	A3	Y W D Q E T R N M K A S A Q T D R V D	DRB10306	D R R G Q
	A4	Y W D E E T R S V K A S A Q T D R V D	DRB10307	D E R A A
	B1	Y W D R E T R N M K A S A Q T D R E N	DRB10308	D E R A A
	B3	Y W D R E T R N V K A S A Q T D R E N	DRB10309	D E R A A
	B2	Y W D R E T Q I S K T N A Q T Y R E S	DRB10201	Q K R G Q
	B4	Y W D R E T Q I S K T N A Q T Y R V G	DRB10102	R R R A A
	B5	Y W D R E T Q I S K T N A Q T Y R E S	DRB10201	Q R R A A

TABLE 6

DNA PRIMERS FOR PCR OF MHC CLASS 1 B ALPHA-1 DOMAIN OF HLA B8, B35, B51, B78

PROTEIN SEQUENCE	**D**	**R**	**N**	**T**	**Q**	**I**	**F**	**K**	**T**	**N**	**T**
	Asp	Arg	Asn	Thr	Gln	Ile	Phe	Lys	Thr	Asn	Thr
DNA SEQUENCES	GAT	AGA	AAT	ACT	CAA	ATT	TTT	AAA	ACU	AAT	ACU
	GAC	AGG	AAC	ACC	CAG	ATC	TTC	AAG	ACC	AAC	ACC
						ATA					
CONSENSUS DNA SEQUENCE	GA*	AG*	AA*	AC*	CA*	AU*	UU*	AA*	AC*	AA*	AC*
	T	A	T	T	A	T	T	G	T	T	T
	C	C	C	C	G	C	C	G	C	C	C
						A					

DNA PRIMERS FOR PCR OF MHC CLASS 1 B ALPHA-1 DOMAIN OF HLA B44 B52 B70/72 B4/40

PROTEIN SEQUENCE	**D**	**R**	**E**	**T**	**Q**	**I**	**S**	**K**	**T**	**N**	**T**
	Asp	Arg	Glu	Thr	Gln	Ile	Ser	Lys	Thr	Asn	Thr
DNA SEQUENCES	GAT	AGA	GAA	ACT	CAA	ATT	AGT	AAA	ACU	AAT	ACU
	GAC	AGG	GAG	ACC	CAG	ATC	AGC	AAG	ACC	AAC	ACC
						ATA					
CONSENSUS DNA SEQUENCE	GA*	AG*	GA*	AC*	CA*	AU*	AG*	AA*	AC*	AA*	AC*
	T	A	A	T	A	T	T	A	T	T	T
	C	G	G	C	G	C	C	G	C	C	C
						A					

TABLE 7

RESPONSE TO VHP1 and HLA PHENOTYPE

Autocytotoxic responses of short term cultures of PBMC from uninfected donors stimulated with VHP1
(in vitro Cr release cytotoxicity assay with autologous target cells)

Peptide added to short term culture	VHP1	Control
DONOR		
Trigger+		
RM	+	-
SW	+	-
WW	+	-
JMC	+	-
Trigger-		
TM	-	-
JF	-	-
KB	-	-
ML	-	-

Trigger: Trigger sequence DRNTQIFKTNT in HLA Class 1 B8 or B35

ABBREVIATIONS

Amino Acid	One Letter Symbol
Alanine	A
Arginine	R
Asparagine	N
Aspartic Acid	D
Asparagine or aspartic acid	B
Cysteine	C
Glutamine	Q
Glutamic acid	E
Glutamine or glutamic acid	Z
Glycine	G
Histidine	H
Isoleucine	I
Leucine	L
Lysine	K
Methionine	M
Phenylalanine	F
Proline	P
Serine	S
Threonine	T
Tryptophan	W
Tyrosine	Y
Valine	V

Peptides

"VHPs" means Viral Homologous Peptides derived from HIV sequences which are homologous to human sequences.

"HEPs" means Human Endogenous Peptides derived from human sequences mimiced by VHPs.

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=-=-=-=

CLAIMS

WHAT IS CLAIMED IS:

1. A preparation which inhibits, partially or completely, autoimmune responses *in vivo* in either HIV infected patients or SLE patients as determined by an *in vitro* autoreactive T cell Proliferation Assay.

2. The preparation of claim 1, wherein said preparation contains at least one or more Non-Steroidal Anti-Inflammatory Drugs and one or more types of peptides.

3. The preparation of claim 2, wherein said Non-Steroidal Anti-Inflammatory Drug is acetyl salicylic acid (aspirin).

4. The preparation of claim 2, wherein said peptides are up to thrity aminoacids long and are derived from protein sequences from any virus but have at least a fifty percent homology to any sequence of a human protein (VHP type of peptide).

5. The preparation of claim 2, wherein said peptides are up to thirty amino acids long and are derived from protein sequences from any human protein but have at least a fifty percent homology to any sequence of a protein from any virus (HEP type of peptide).

6. The preparation of claim 2, wherein said peptides are up to thirty amino acids long and are derived both from protein sequences from any virus but have at least a fifty percent homology to any sequence of a human protein (VHP type of peptide) and from protein sequences from any human protein but have at least a fifty percent homology to any sequence of a protein from any virus (HEP type of peptide).

7. The preparation of claim 4, wherein said virus is Human Immunodeficiency Virus (HIV).

8. The preparation of claim 5, wherein said virus is Human Immunodeficiency Virus (HIV).

9. The preparation of claim 6, wherein said virus is Human Immunodeficiency Virus (HIV).

10. The preparation of claim 7, wherein the said peptide contains the sequence TKAKRR

12. The preparation of claim 9, wherein the said VHP peptide contains the sequence TKAKRR

13. The preparation of claim 7, wherein the said peptide is VHP0

14. The preparation of claim 7, wherein the said peptide is VHP1

15. The preparation of claim 7, wherein the said peptide is VHP2

16. The preparation of claim 7, wherein the said peptide is VHP3

17. The preparation of claim 7, wherein the said peptide is VHP4

18. The preparation of claim 7, wherein the said peptide is VHP5

19. The preparation of claim 7, wherein the said peptide is VHPo

20. The preparation of claim 7, wherein the said peptide is VHP7

21. The preparation of claim 8, wherein the said peptide is HEP1

22. The preparation of claim 8, wherein the said peptide is HEP2

23. The preparation of claim 8, wherein the said peptide is HEP3

24. The preparation of claim 8, wherein the said peptide is HEP4

25. The preparation of claim 8, wherein the said peptide is HEP5

26. The preparation of claim 8, wherein the said peptide is HEP6

27. The preparation of claim 8, wherein the said peptide is HEP7

28. The preparation of claim 8, wherein the said peptide is HEP8

29. The preparation of claim 8, wherein the said peptide is HEP9

30. The preparation of claim 8, wherein the said peptide is HEP10

31. The preparation of claim 8, wherein the said peptide is HEP11

32. The preparation of claim 8, wherein the said peptide is HEP12

33. The preparation of claim 8, wherein the said peptide is HEP13

34. The preparation of claim 8, wherein the said peptide is HEP14

36. The preparation of claim 8, wherein the said peptide is HEP15

37. The preparation of claim 8, wherein the said peptide is HEP16

38. The preparation of claim 8, wherein the said peptide is HEP17

39. The preparation of claim 8, wherein the said peptide is HEP18

40. The preparation of claim 8, wherein the said peptide is HEP19

41. The preparation of claim 8, wherein the said peptide is HEP20

42. The preparation of claim 8, wherein the said peptide is HEP22

43. The preparation of claim 8, wherein the said peptide is HEP23

44. The preparation of claim 8, wherein the said peptide is HEP24

45. The preparation of claim 8, wherein the said peptide is HEP25

46. The preparation of claim 1, wherein said preparation contains at least one or more types of peptides.

47. The preparation of claim 46, wherein said peptides are up to thirty amino acids long and are derived from protein sequences from any virus but have at least a fifty percent homology to any sequence of a human protein (VHP type of peptide).

48. The preparation of claim 46, wherein said peptides are up to thirty amino acids long and are derived from protein sequences from any human protein but have at least a fifty percent homology to any sequence of a protein from any virus (HEP type of peptide).

49. The preparation of claim 46, wherein said peptides are up to thirty amino acids long and are derived both from protein sequences from any virus but have at least a fifty percent homology to any sequence of a human protein (VHP type of peptide).and from protein sequences from any human protein but have at least a fifty percent homology to any sequence of a protein from any virus (HEP type of peptide).

50. The preparation of claim 47, wherein said virus is Human Immunodeficiency Virus (HIV).

51. The preparation of claim 48, wherein said virus is Human Immunodeficiency Virus (HIV).

52. The preparation of claim 49, wherein said virus is Human Immunodeficiency Virus (HIV).

53. The preparation of claim 50, wherein the said peptide contains the sequence TKAKRR

54. The preparation of claim 52, wherein the said VHP peptide contains the sequence TKAKRR

55. The preparation of claim 50, wherein the said peptide is VHP0

56. The preparation of claim 50, wherein the said peptide is VHP1

57. The preparation of claim 50, wherein the said peptide is VHP2

58. The preparation of claim 50, wherein the said peptide is VHP3

59. The preparation of claim 50, wherein the said peptide is VHP4

60. The preparation of claim 50, wherein the said peptide is VHP5

61. The preparation of claim 50, wherein the said peptide is VHP6

62. The preparation of claim 50, wherein the said peptide is VHP7

63. The preparation of claim 51, wherein the said peptide is HEP1

64. The preparation of claim 51, wherein the said peptide is HEP2

65. The preparation of claim 51, wherein the said peptide is HEP3

66. The preparation of claim 51, wherein the said peptide is HEP4

67. The preparation of claim 51, wherein the said peptide is HEP5

68. The preparation of claim 51, wherein the said peptide is HEP6

69. The preparation of claim 51, wherein the said peptide is HEP7

70. The preparation of claim 51, wherein the said peptide is HEP8

71. The preparation of claim 51, wherein the said peptide is HEP9

72. The preparation of claim 51, wherein the said peptide is HEP10

73. The preparation of claim 51, wherein the said peptide is HEP11

74. The preparation of claim 51, wherein the said peptide is HEP12

75. The preparation of claim 51, wherein the said peptide is HEP13

76. The preparation of claim 51, wherein the said peptide is HEP14

77. The preparation of claim 51, wherein the said peptide is HEP15

78. The preparation of claim 51, wherein the said peptide is HEP16

79. The preparation of claim 51, wherein the said peptide is HEP17

80. The preparation of claim 51, wherein the said peptide is HEP18

81. The preparation of claim 51, wherein the said peptide is HEP19

82. The preparation of claim 51, wherein the said peptide is HEP20

83. The preparation of claim 51, wherein the said peptide is HEP22

84. The preparation of claim 51, wherein the said peptide is HEP23

85. The preparation of claim 51, wherein the said peptide is HEP24

86. The preparation of claim 51, wherein the said peptide is HEP25

87. A diagnostic technique for the detection of proteins in an assay sample containing any of the following protein sequences:

a) DRNTQIFKTNT

b) DRETQISKTNT

88. A technique of claim 87 comprising of bringing the assay sample into contact with an assay substrate and sudsequently assaying binding of said substrate.

89 The technique of claim 88 wherein the substrate is an antibody, said antibody being labelled, excess of said antibody being removed before assay, said assay being to detect said label.

90 The technique of claim 88, wherein the said assay comprises FACS analysis or fluorescence microscopy.

91. The technique of claim 88, wherein said assay is selected from a group consisting of: enzyme-linked immnuosorbent assay; one step radioimmuneassay; two step radioimmuneassay; and sandwich radioimmuneassay.

92. The technique of claim 88, wherein said substance is labeled , a label selected from the group consisting of ^{125}I, ^{121}I, ^{14}C, ^{35}S, ^{3}H, ^{112}In, ^{99m}Tc. fluorescien, rhodamine, biotin, horseradish peroxidase or alkaline phosphatase or phycerythrin.

93. A kit comprising the essentisal components of any of the techniques defined in claim 88.

94 A kit comprising of at least a substrate as defined in claim 88, and wherein said substrate is bound to a support thereof

95 An antibody of claim 89, wherein the antibody is raised by the following method comprising tolerising a mammal, with resting T cells prepared from at least one individual having an MHC Class I B haplotype associated with AIDS-sensitivity and, thereafter, exposing the immune system of the animal to an immunogenic dose of activated T cells from at least one individual known to have an MHC Class I B haplotype associated with AIDS-sensitivity, the individual in each case preferably being the same, both for the tolerising and the immunogenic doses, and thereafter preparing the cell line in accordance with known techniques.The activated T cells may be activated by a known technique for example. One technique involves exposing the cells to a dose phytohaemagglutinin, or other suitable toxin.

95 An antibody of claim 89, wherein the antibody is raised by the following method comprising tolerising a mammal, with resting T cells prepared from at least one individual having an MHC Class I B haplotype associated with AIDS-resistance and, thereafter, exposing the immune system of the animal to an immunogenic dose of activated T cells from at least one individual known to have an MHC Class I B haplotype associated with AIDS-resistance, the individual in each case preferably being the same, both for the tolerising and the immunogenic doses, and thereafter preparing the cell line in accordance with known techniques.The activated

T cells may be activated by a known technique for example. One technique involves exposing the cells to a dose phytohaemagglutinin, or other suitable toxin.

96 An antibody of claim 89, wherein the antibody is monoclonal.

97 An antibody of claim 89, wherein the antibody is monoclonal antibody LA45

98 A technique of claim 87, comprising of bringing an assay sample into contact with an assay substrate and sudsequently assaying said proteins by detecting with DNA or RNA coding for the said protein by the Polymerise Chain Reaction method using suitable oligonucleotide primers.

99 Oligonucleotide primers of claim 98, wherein the primer is identified in Table 6.

Amendments to the claims have been filed as follows

1. Composition of matter comprising a purified peptide or mixture of two or more different peptides up to thirty amino acids long or derivative molecules with additional chemical groups attached to such peptides, comprising an amino acid sequence with at least 50% homology over fourteen consecutive amino acids with the following amino acid sequence:
NH_2ThrGluLysLysArgArgGluThrValGluArgGluLysGluCOOH,
(the fourteen amino acid sequence hereinafter referred to as HEP1) and a purified peptide or mixture of two or more different peptides up to thirty amino acids long or derivative molecules with additional chemical groups attached to such peptides, comprising an amino acid sequence of at least five consecutive amino acids with 100% homology with HEP1.

2. A purified peptide of claim 1 comprising an amino acid sequence sufficiently duplicative of the fourteen amino acid sequence HEP1.

3. A preparation of claim 1 for the prevention and treatment of AIDS and related disorders which inhibits *in vivo* in man , partially or completely, HIV virus as measured by either an HIV p24 antigen assay *in vitro* or by an HIV infectivity assay *in vitro.*

4. A preparation of claim 1 for the prevention and treatment of AIDS and related disorders, and for the prevention and treatment of Systemic Lupus Erythematosus and related disorders, which inhibits *in vivo* in man, partially or completely, autoimmune or autoreactive responses measured *in vitro* in a T cell Proliferation Assay.

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